Endothelin and cardiovascular remodelling in end-stage renal disease

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Abstract

Background. Plasma endothelin (ET) is elevated in end-stage renal disease (ESRD), but the origin and consequences of this increase remain unclear. In the present study we analysed the relationships between plasma ET levels and cardiovascular alterations in ESRD.

Methods and results. Common carotid artery (CCA) intima–media thickness (IMT) and diameter, atherosclerotic plaque occurrence, and left ventricular (LV) geometry and function were determined by ultrasound imaging in 76 haemodialysis patients and in 57 age-, sex-, and blood pressure-matched controls. Arterial stiffness was evaluated via carotid–femoral pulse wave velocity (CF–PWV), forearm post-ischaemic vasodilation was measured by venous plethysmography, and plasma ET levels were determined using a specific immunoenzymoassay. Compared with controls, ESRD patients had elevated plasma ET levels (1.6 ± 1.4 vs 4.6 ± 3.8 pg/ml; P < 0.001), increased LV mass (P < 0.001), increased CCA–IMT (P < 0.001), a higher prevalence of atherosclerotic plaques (P < 0.001) and increased CF–PWV (P < 0.01). Plasma ET levels correlated positively with LV outflow velocity integral (r = 0.57; P < 0.001), stroke index (P < 0.01), and baseline forearm blood flow (P < 0.001) which were all significantly higher in ESRD patients than in controls (P < 0.01). After adjustment for age, blood pressure, haemoglobin levels, gender and body dimensions, plasma ET levels were significantly correlated to LV mass (r = 0.46; P < 0.001), CCA–IMT and CCA intima–media cross-sectional area (r = 0.41; P < 0.001), and CF–PWV (P < 0.05). Post-ischaemic forearm vasodilation was decreased in ESRD (85 ± 31 vs 119 ± 28%; P < 0.001) and there was a negative correlation between post-ischaemic flow recovery and ET levels (r = −0.49; P < 0.001). In ESRD patients, plasma ET levels were positively and independently correlated with the prevalence of CCA atherosclerotic plaque (P < 0.01).

Conclusions. These results indicate that the increased plasma ET levels in ESRD patients are associated with left ventricular hypertrophy and arterial intima–media thickening, suggesting that increased ET concentrations in ESRD patients may be of pathophysiological significance in the process of cardiovascular remodelling.

Key words: endothelin; ESRD; cardiovascular remodelling

Introduction

Cardiovascular disease is the major cause of death and morbidity in end-stage renal disease (ESRD) patients [1]. The principal structural alterations associated with cardiovascular complications are left ventricular hypertrophy (LVH), dilatation and hypertrophy of large conduit arteries, and occlusive atherosclerotic lesions [2–5]. Structural alterations of the cardiovascular system occur principally in response to long-term pressure and/or flow overload, and also from the interaction between these mechanical stimuli and locally generated growth factors and vasoactive substances.

Endothelin (ET) is a potent vasoconstrictor which occurs in three isoforms; ET-1, ET-2, and ET-3, with plasma levels corresponding predominantly to ET-1. While in normal conditions ET acts essentially as a paracrine/autocrine factor, in several pathological conditions ET could be also considered as a circulating ‘hormone’ [6,7]. ET is also a mitogen that stimulates cardiac hypertrophy and vascular smooth muscle proliferation [8–10]. Plasma ET levels are low in normal subjects, but augmented in several disease states such as congestive heart failure, acute myocardial infarction, atherosclerosis, liver cirrhosis, and renal failure [11–20]. ET is particularly elevated in haemodialysis patients, but the mechanisms responsible for this increase are not fully understood, and its pathophysiological significance remains unclear [17–20].

The present study was designed to analyse in patients with ESRD the relationships between plasma ET levels and cardiovascular remodelling and functional alterations.

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Subjects and methods

Subjects

Stable, non-diabetic, ESRD patients (n=76) on haemodialysis for 105±85 months (6–312 months) were included. Age-, sex-, and blood pressure (BP)-matched non-uremic subjects (n=57) served as the control group. On the basis of clinical report and complementary paraclinical investigations (echocardiography, ECG, echodoppler study) patients or controls with acute myocardial infarction, valvular heart disease, cerebral vascular disease, common carotid artery stenosis, and heart failure (according to American Heart Association criteria) were excluded. ESRD patients (n=50) were being treated with regular recombinant human erythropoietin. In all, 22 ESRD patients and 15 controls were taking antihypertensive therapy, which was stopped 4 weeks prior to the study. Seven ESRD patients had had binephrectomy performed 168–300 months before the study. Patients were dialysed with an AN69 membrane (Hospal, Meyzieu, France) with bicarbonate dialysate. The duration of dialysis was individually tailored (4–6 h thrice weekly) to control body fluids and blood chemistry, and to achieve a Kt/V=1.2 (1.52±0.15). Investigations were performed in the morning before the mid-week haemodialysis session. Each subject provided informed written consent for the study, which was approved by our institutional review board.

Cardiac measurements

Echocardiographic studies were performed using a Hewlett-Packard Sonos 100 device (HP, Eivy, France) equipped with a 2.25 MHz probe allowing M-mode, two-dimensional, and pulsed Doppler measurements. Measurements were performed blindly by two physicians according to the American Society of Echocardiography [22]. M-mode measurements included left ventricular (LV) posterior wall thickness (PWT), interventricular septal thickness (IVS), LV end-diastolic diameter (LVEDD), and LV end-systolic diameter (LVESD). LV mass was calculated according to the Penn convention [23], and LV mean wall thickness as (IVS+PWT)/2. Fractional shortening was calculated as ((LVEDD–LVESD)/LVEDD)×100. LV outflow and aortic velocities were taken from the apical position, and mitral inflow velocities with the signal positioned at the tip of the mitral leaflets.

Blood pressure (BP) and arterial measurements

Brachial BP was measured with a mercury sphygmomanometer after 15 min of recumbency. Phases I and V of the Korotkoff sounds were taken respectively as the systolic BP and diastolic BP. The mean BP (MBP) was calculated as: MBP= DBP + (SBP–DBP)/3. Five measurements 2 min apart were averaged.

Common carotid artery diameter and intima–media thickness

Common carotid artery (CCA) diastolic diameter (CCAD) was measured by a high-resolution B-mode (7.5 MHz transducer) echotracking system (Wall-Track system: W-T, Maastricht, The Netherlands). A complete detailed description of this system and the reproducibility of the measurements has been published previously [4,23]. The accuracy of the system is ±30 μm for CCAD. The repeatability coefficient of the measurements was ±0.273 mm for CCAD. Measurements were done on the right CCA, two centimeters beneath the bifurcation. CCA intima–media thickness (IMT) was measured on the far wall at the same level as the diameter measurements with computer-assisted acquisition, processing and storage. The computing equipment was linked to an 80386/16 MHz processor and an imaging card providing real-time digitizing of the video analogue signal from the echo recording (processing corresponding to 256 levels of gray). The IMT was automatically analysed from changes in density on the section perpendicular to the vessel wall with dedicated software (Eurequa; TSA, Meudon, France) [24]. The repeatability coefficient of the IMT measurement was ±60 μm [4]. The CCA intima-media cross-sectional area was calculated as π(CCAD 2 /IMT 2 )–π(CCAD 2 /2). A localised echostructure encroaching into the vessel lumen was considered to be a plaque if the CCA-IMT was >50% thicker than neighbouring sites. A semiquantitative scale was used to assess plaque occurrence: grade 1 = no plaque, and grade 2 = plaque present. CCAD and CCA-IMT measurements were always performed in plaque-free arterial segments.

Arterial stiffness

The carotid–femoral PWV (CF–PWV) was determined using the foot-to-foot method [4,25]. CCA and femoral artery pressure waveforms were recorded non-invasively with a pencil-type probe incorporating a high-fidelity Millar strain gauge transducer in the tip of the probe (SPT-301; Millar Instruments, Houston, TX, USA). Pressure waveform recordings were carried out simultaneously at the base of the neck over the CCA and the femoral artery in the groin and recorded with a Gould 8188 recorder (Gould Electronique, Ballainvilliers, France) at a speed of 100 mm/s. The time delay (t) was measured between the feet of the pressure waves recorded at these different points. The time delay was averaged over 10–15 beats. The distance travelled by the pulse wave (D) was measured over the body surface as the distance between the two recording sites minus that from the suprasternal notch to the carotid. PWV was calculated as PWV = D/t. Left ventricular ejection time (LVET) and heart period were determined from CCA pressure wave records as previously described [4].

Forearm reactive hyperaemia

Forearm blood flow (FBF in ml/100ml/min) was measured by venous occlusion plethysmography (on the forearm contralateral to the arteriovenous shunt in ESRD) in 40 control subjects and 49 ESRD patients. The forearm was placed in adduction 10 cm above heart level. A pneumatic venous occlusion cuff was placed around the upper arm and connected to an automatic inflator. A mercury strain gauge was placed on the largest part of the arm distal to the humeral epicondyle. After calibration, venous occlusion was achieved by cuff inflation to 50 mm Hg over 2 s for the measurement of baseline blood flow. The reactive hyperaemia response of the forearm was assessed using a standardized protocol [26] (Figure 1). In addition to the previously described apparatus, a second pneumatic cuff (arterial occlusion cuff) was placed on the arm proximal to the venous occlusion cuff and the gauge. After control flow measurements were obtained, the arterial occlusion cuff was inflated to 300 mm Hg for a period of 5 min. The venous occlusion cuff was inflated to
Endothelin and cardiovascular remodelling in ESRD

Fig. 1. Schematic representation of reactive hyperaemia response in the human forearm after 5 min of ischaemia (ref. 27). Flow debt repayment (B/A in %) is the ratio between excess hyperaemic flow (B=surface under the curve between the release of ischaemia and duration of hyperaemia) and the flow debt (A=surface under the curve between the start and end of ischaemic period).

50 mm Hg 10 s before deflating the arterial cuff. At time 'zero' the arterial cuff was deflated and the FBF measured. FBF was then measured every 15 s until resting flow levels were reached, and then every minute until a total post-ischaemic period of 5 min had elapsed. The following parameters were determined: the flow debt (integral of resting flow over arterial occlusion time), the post-ischaemic peak flow (% of baseline), the duration of the hyperaemic phase (s), the excess hyperaemic flow (integral of flow and duration of hyperemic response), and the debt repayment ratio (excess hyperaemic flow/flow debt). All measurements were made with a wrist cuff inflated 20 mm Hg above the SBP.

Laboratory methods

Arteriovenous fistula blood was obtained immediately before haemodynamic investigations after an overnight fast. The plasma was separated without delay at 4 °C in a refrigerated centrifuge, and stored at −20 °C until plasma ET determination was performed using an enzyme immunoassay kit (Cayman Chemicals Company, Ann Arbor, MI, USA) after an extraction procedure. Briefly, plasma was acidified with 4% acetic acid, and immunoreactive endothelin was extracted with a Sep-pac C 18 cartridge (Waters Associates, Milford, MA, USA). To determine the recovery rate, a sample was spiked with a known amount of ET, then acidified and treated in serial. Each column was pretreated by sequentially adding methanol, distilled water and 4% acetic acid; the acidified sample was then added. After washing with 25% ethanol, the adsorbed peptide was eluted with 86% ethanol in 4% acetic acid. After elute evaporation, the dry residue was dissolved in assay buffer and subjected to the EIAs. The recovery rate was 60 ± 3%. The immunometric assay is based on a double antibody 'sandwich' technique. Each well of the microtitre plate is coated with a monoclonal antibody specific for human ET (ET capture antibody). The second antibody is a monoclonal antibody labelled with an acetyl cholinesterase (AChE) and is selective for a different epitope on the ET molecule. The concentration of the analyte is then determined by measuring the enzymatic activity of the AChE with Ellman's reagent. The assay for ET did not cross-react with big ET. While assay specificity is 100% for ET-1, ET-2 and ET-3, the plasma levels reflect principally plasma ET-1 concentrations [27]. The ET detection threshold is 0.1 pg/ml. The inter- and intra-assay coefficients of variation were <10%. Routine biochemical parameters were determined by standard methods, and serum albumin and plasma fibrinogen by the nephelometric method.

Statistical analysis

Data were expressed as means ± SD. Student’s t-test was used for comparison of controls and ESRD patients. Qualitative data were compared using the χ² test. Gender was used as a dummy variable (1 = male, 2 = female). Multiple stepwise regression analysis was used to assess the correlations between plasma ET levels and determinants of cardiac and arterial parameters and interactions. P < 0.05 was considered to be significant.

Results

Study population

Population characteristics are presented in Tables 1–3. The two groups were similar regarding age, sex ratio,
SBP and DBP. Pulse pressure was increased in ESRD. A total of 21 control subjects (37%) and 42 ESRD patients (55%) (not significant) had a past history of hypertension, and 15 controls and 22 ESRD patients were being treated with antihypertensive drugs (stopped 4 weeks before the study). ESRD patients had lower body height and weight (P < 0.001), and lower body mass index (P < 0.001). The ankle/arm systolic pressure index was similar in the two groups.

Cardiovascular parameters

LV diameter, wall thickness, and mass were increased in ESRD patients (P < 0.001) (Table 2). LV outflow velocity integral (LVoVI), maximum aortic flow velocity, and stroke index were increased in ESRD (P < 0.001), and inversely correlated with plasma haemoglobin (P < 0.01). Mitral E/A ratio and fractional shortening were lower in ESRD (P < 0.001 and P < 0.05). ESRD patients had a shorter heart period (P < 0.001 and LVET (P < 0.05). CCAD and IMT were increased in ESRD (P < 0.001). ESRD patients had a higher carotid–femoral PWV (P < 0.01) and a higher prevalence of atherosclerotic plaque (P < 0.001). In ESRD patients, pulse pressure was positively correlated with LVoVI (P < 0.001) and CF–PWV (P < 0.001). Baseline FBF was increased in ESRD patients (P < 0.05) (Table 2) and was positively correlated with LVoVI (P < 0.001) and stroke index (P < 0.01). In ESRD patients, the duration of post-ischaemic vasodilatation was shorter (106 ± 29 vs 132 ± 36 s, P < 0.001), with decreased flow debt repayment (P < 0.001) and lower post-ischaemic peak flow (P < 0.01). Post-ischaemic vasodilatation was negatively correlated with age and CCA–IMT (P < 0.01).

Blood chemistry and endothelin in ESRD

ESRD patients had lower total and high density lipoprotein (HDL) cholesterol (P < 0.05 and P < 0.001) and increased triglycerides (P < 0.001) (Table 3). Serum albumin was lower in ESRD patients (P < 0.01), and was negatively correlated with age (P < 0.001) and fibrinogen levels (P < 0.01). Plasma fibrinogen was increased in ESRD patients (P < 0.001) and was positively correlated with age (r = 0.49; P < 0.01).

Compared with controls (1.6 ± 1.4; range 0.1–3.7 pg/ml), plasma ET was increased in ESRD (4.6 ± 3.8; range 0.2–18.7 pg/ml; P < 0.001) (Table 3), and increased with age (P < 0.05). ET concentration was similar in binephrectomized patients and those with native kidneys (4.2 ± 2.1 vs 5.0 ± 4.2 pg/ml; NS). After adjustment for age, ET concentrations were not correlated with systolic or diastolic BP, but correlated significantly with pulse pressure (r = 0.50; P < 0.001). Independently from age, pulse pressure and haemoglobin levels, plasma ET was positively correlated with LVoVI (r = 0.57; P < 0.0001) (Figure 2), maximum aortic flow velocity (r = 0.42; P < 0.001), stroke index (r = 0.31; P < 0.01), and baseline FBF (r = 0.49; P < 0.001).

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**Table 1.** Clinical characteristics. Values are shown as mean ± SD

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Controls (n = 57)</th>
<th>ESRD (n = 76)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>49.2 ± 14.7</td>
<td>51.6 ± 15.5</td>
</tr>
<tr>
<td>Sex (M/F ratio)</td>
<td>1.35 ± 0.48</td>
<td>1.40 ± 0.49</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>170.8 ± 10.7</td>
<td>164.0 ± 11.0</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>76.1 ± 16.1</td>
<td>61.7 ± 13.5</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>26.0 ± 4.7</td>
<td>23.0 ± 3.9</td>
</tr>
<tr>
<td>Systolic BP (mm Hg)</td>
<td>145.0 ± 15.5</td>
<td>149.0 ± 28.4</td>
</tr>
<tr>
<td>Diastolic BP (mm Hg)</td>
<td>85.0 ± 14.8</td>
<td>83.5 ± 15.1</td>
</tr>
<tr>
<td>Mean BP (mm Hg)</td>
<td>104.5 ± 14.2</td>
<td>105.9 ± 17.5</td>
</tr>
<tr>
<td>Pulse pressure (mm Hg)</td>
<td>59.4 ± 15.5</td>
<td>65.9 ± 21.8</td>
</tr>
<tr>
<td>Ankle/arm pressure index</td>
<td>1.10 ± 0.08</td>
<td>1.12 ± 0.08</td>
</tr>
</tbody>
</table>

*P < 0.05; ***P < 0.001.

**Table 2.** Cardiac and arterial measurements. Values are shown as mean ± SD

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Controls</th>
<th>ESRD</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV end-diastolic diameter (cm)</td>
<td>5.10 ± 0.50</td>
<td>5.43 ± 0.60***</td>
</tr>
<tr>
<td>LV mean wall thickness (cm)</td>
<td>0.92 ± 0.12</td>
<td>1.06 ± 0.19***</td>
</tr>
<tr>
<td>LV mass (g)</td>
<td>201 ± 60</td>
<td>278 ± 102***</td>
</tr>
<tr>
<td>LV mass index (g/m²)</td>
<td>107 ± 24</td>
<td>164 ± 54***</td>
</tr>
<tr>
<td>Percentage shortening</td>
<td>38.0 ± 5.5</td>
<td>35.7 ± 5.7</td>
</tr>
<tr>
<td>E/A (ratio)</td>
<td>1.29 ± 0.36</td>
<td>0.94 ± 0.32***</td>
</tr>
<tr>
<td>Heart period (ms)</td>
<td>962 ± 149</td>
<td>862 ± 136***</td>
</tr>
<tr>
<td>LV ejection time (ms)</td>
<td>308 ± 29</td>
<td>296 ± 33**</td>
</tr>
<tr>
<td>LV outflow velocity integral (cm²/beat)</td>
<td>22.0 ± 4.1</td>
<td>27.6 ± 7.5***</td>
</tr>
<tr>
<td>Maximum aortic flow velocity</td>
<td>110 ± 17</td>
<td>127 ± 25***</td>
</tr>
<tr>
<td>(cm/s)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stroke index (ml/m²)</td>
<td>45.0 ± 6.6</td>
<td>51.7 ± 1.0***</td>
</tr>
<tr>
<td>CCA diameter (mm)</td>
<td>5.67 ± 0.65</td>
<td>6.40 ± 0.90***</td>
</tr>
<tr>
<td>CCA intima–media thickness (µm)</td>
<td>710 ± 93</td>
<td>790 ± 110***</td>
</tr>
<tr>
<td>Carotid–femoral PWV (cm/s)</td>
<td>962 ± 187</td>
<td>1070 ± 242***</td>
</tr>
<tr>
<td>Forearm blood flow (ml/min/100 ml)</td>
<td>3.4 ± 1.3</td>
<td>4.1 ± 1.6*</td>
</tr>
<tr>
<td>Flow debt repayment (%)</td>
<td>119 ± 28</td>
<td>85 ± 31***</td>
</tr>
<tr>
<td>Peak flow (% of baseline)</td>
<td>892 ± 347</td>
<td>691 ± 303**</td>
</tr>
<tr>
<td>CCA atherosclerosis (ratio)*</td>
<td>1.19 ± 0.40</td>
<td>1.54 ± 0.45***</td>
</tr>
</tbody>
</table>

*Plaques: 1 = absent; 2 = present. LV = left ventricular; E/A = transmural early-to-late peak velocity; CCA = common carotid artery; PWV = pulse wave velocity. *P < 0.05; **P < 0.01; ***P < 0.001.

**Table 3.** Blood chemistry. Values are given as mean ± SD

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Controls</th>
<th>ESRD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin (g/dl)</td>
<td>14.9 ± 1.6</td>
<td>11.1 ± 1.9***</td>
</tr>
<tr>
<td>Blood urea (mmol/l)</td>
<td>6.1 ± 1.7</td>
<td>24.0 ± 3.2***</td>
</tr>
<tr>
<td>Creatinine (mmol/l)</td>
<td>0.10 ± 0.01</td>
<td>0.90 ± 0.13***</td>
</tr>
<tr>
<td>Serum albumin (g/l)</td>
<td>44.7 ± 2.6</td>
<td>39.9 ± 3.0**</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>5.02 ± 0.48</td>
<td>4.84 ± 0.56</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>5.28 ± 1.04</td>
<td>4.91 ± 1.06*</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>1.38 ± 0.39</td>
<td>1.09 ± 0.35***</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>1.39 ± 0.63</td>
<td>1.87 ± 0.96***</td>
</tr>
<tr>
<td>Plasma fibrinogen (g/l)</td>
<td>3.21 ± 0.78</td>
<td>4.75 ± 1.04***</td>
</tr>
<tr>
<td>Smoking status (packet.years)</td>
<td>10.5 ± 17.6</td>
<td>11.0 ± 17.3</td>
</tr>
<tr>
<td>Plasma endothelin (pg/ml)</td>
<td>1.60 ± 1.40</td>
<td>4.60 ± 3.80***</td>
</tr>
</tbody>
</table>

*P < 0.05; ***P < 0.001.
An age-independent, inverse correlation was observed between plasma ET and post-ischaemic flow debt repayment ($r = -0.36; P < 0.01$) and post-ischaemic peak flow ($r = -0.49; P < 0.001$) (Figure 3). After adjustment for body surface area, blood pressure, haemoglobin level, gender, and age, LV mass was positively correlated with plasma ET concentration (Table 4) (Figure 4). Significant blood pressure-, age-, gender-, and haemoglobin-adjusted positive correlation was also observed between ET levels and CCA-IMT or intima-media cross-sectional area ($r = 0.41; P < 0.001$) (Figure 4). In ESRD, CCA-IMT was positively correlated with CF-PWV ($r = 0.55; P < 0.0001$). After adjustment for age, blood pressure and CCA-IMT, the positive correlation between plasma ET levels and CF-PWV persisted ($r = 0.23; P < 0.05$). Plasma ET concentration was not correlated with the dose of erythropoietin received by ESRD patients ($r = 0.137; P = 0.227$), nor with blood urea, creatinine, blood lipid, serum albumin or plasma fibrinogen level abnormalities, nor with smoking habits.

Atherosclerotic plaque occurrence was associated...
Table 4. Multiple step-wise regression analysis in ESRD patients

<table>
<thead>
<tr>
<th>Parameters</th>
<th>T value</th>
<th>Sequential P value</th>
<th>Simple r²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body surface area (m²)</td>
<td>3.8</td>
<td>0.2807</td>
<td>0.0003</td>
</tr>
<tr>
<td>Systolic BP (mm Hg)</td>
<td>2.6</td>
<td>0.3891</td>
<td>0.0116</td>
</tr>
<tr>
<td>Plasma endothelin (pg/ml)</td>
<td>2.6</td>
<td>0.4654</td>
<td>0.0119</td>
</tr>
<tr>
<td>Haemoglobin (g/dl)</td>
<td>−2.7</td>
<td>0.5160</td>
<td>0.0092</td>
</tr>
<tr>
<td>Gender (1 = male; 2 = female)</td>
<td>−2.3</td>
<td>0.5057</td>
<td>0.0251</td>
</tr>
</tbody>
</table>

Dependent variable: carotid plaques; (1 = absent; 2 = present)

Plasma endothelin (pg/ml) 3.2 0.2312 0.0023 0.2312
Plasma fibrinogen (g/l) 2.8 0.4122 0.0071 0.2985
Smoking status (packet.year) 2.4 0.4712 0.0214 0.1506
Age (years) 2.1 0.5064 0.0447 0.2465

r² = 0.5064; F = 15.13; (P < 0.0001)

Discussion

The present data confirm previous reports that patients with ESRD have increased plasma ET levels. They extend the previous findings by demonstrating that the elevated plasma ET levels in ESRD patients are significantly correlated with: (i) haemodynamic alterations such as increased blood flow and flow velocity and increased pulse pressure; and (ii) cardiovascular remodelling, including increased LV mass and CCA–IMT, and a higher prevalence of atherosclerotic plaque. These findings are independent of age, gender, body size, systemic BP and the usual biochemical abnormalities observed in chronic uraemia.

The elevated plasma ET concentration in ESRD patients may be due to increased production and release, decreased metabolic clearance, or both. Renal ET production is increased in experimental uraemia and renal ET gene expression increases with progression of renal insufficiency [28,29]. This is consistent with observations in humans that urinary excretion of ET is increased in chronic renal failure [30]. However, with some exceptions [20,31,32], plasma ET concentrations have not been found to correlate significantly with blood urea or creatinine levels in most studies of patients with renal failure [16,17,33,34]. Since kidneys are implicated in the clearance of ET [35], decreased metabolic clearance may contribute to the elevation of circulating ET levels in ESRD patients [34]. In experimental conditions, bilateral nephrectomy caused a modest but significant delay in the disappearance rate of exogenous ET [36]. The fact that in the present study ET levels were similar in binephrectomized patients and in those with native kidneys argues against the kidneys being the only major source of plasma ET in haemodialysis patients and also suggests that decreased renal clearance had limited influence.

Haemodynamic factors, including shear stress and pulsatile tensile stress [37–42] alter the production and release of ET by endothelial cells in vitro. Increased systemic and regional blood flow, and increased pulsatile pressure are characteristic features observed in stable haemodialysis patients [4,43–46], and the present results indicate that these blood flow and pressure alterations are associated with increased plasma levels of ET in ESRD patients. The influence of elevated blood flow on plasma ET concentration in ESRD patients is consistent with the observation of Wilkie et al. [18], who showed that the ET concentration is significantly higher in haemodialysis patients with arteriovenous fistulas than in those dialysed with venous catheters or those treated by peritoneal dialysis. The correlations between ET concentration and cardiac or circulatory function in haemodialysis patients are opposed to those observed in non-uraemic humans with heart failure. In these patients the ET concentration increases in proportion to left ventricular dysfunction, being negatively associated with cardiac index and ejection fraction [11,12]. These paradoxical findings illustrate the complexity of mechanisms related to ET release in vivo.

In ESRD patients the cardiovascular system

![Fig. 4. Scatterplots in ESRD patients showing the positive association between plasma endothelin levels and left ventricular mass (left panel) and common carotid artery intima–media cross-sectional area (CCAMCSA) (right panel).](image-url)
undergoes significant cardiovascular remodelling characterized by an increased left ventricular mass and wall-hypertrophy of major arteries [2–4]. This cardiovascular remodelling results principally from the combination of chronic volume and pressure overload. Volume overload is principally due to anaemia, arteriovenous shunts and overhydration, while pressure overload is mainly due to increased arterial stiffness and early arterial wave reflections [2,4,43,46]. A significant association between plasma ET concentration and LV mass and CCA IMT was observed in ESRD patients. As plasma ET concentration was positively correlated with increased blood flow and flow velocity and increased pulsatile load in central arteries the correlation between ET levels and LV mass and CCA IMT could be due to common influence of haemodynamic overload both on cardiovascular structures and ET release. Nevertheless, ET has a mitogenic action, stimulating growth and proliferation in a variety of cells such as smooth muscle cells, endothelial cells, fibroblasts and cardiomyocytes [8–10] and ET receptors are present on myocardial cells [47,48]. Myocardial ET expression is also induced in response to pressure and volume overload and both cardiac ET production and myocardial ET receptor density are elevated in chronic heart failure [7,11,12,49–52]. Thus, haemodynamic abnormalities observed in ESRD patients could primarily alter the ET release which may in turn directly influence the structure and function of the cardiovascular system.

Lerman et al. [14] found a significant correlation between plasma ET concentrations and the number of sites involved by atherosclerotic vascular disease in non-uraemic subjects. The association between atherosclerosis and ET was also demonstrated by Jones et al. [53], who found that ET-1 is increased overlying atherosclerotic plaques in human arteries. In contrast to the observations of Mallamaci et al. (20), who did not find any influence of cardiovascular damage on plasma ET levels in chronic renal failure, the present study shows that in ESRD patients the prevalence of atherosclerotic plaques is correlated with plasma ET levels independently from other risk factors such as plasma fibrinogen level, smoking habits or age (Table 4). The high plasma fibrinogen and lower serum albumin concentrations observed in ESRD patients were not correlated with ET levels and may be linked to acute phase reaction associated with haemodialysis [54]. Activation of ET could occur as a secondary phenomenon in atherosclerosis, possibly related to atherosclerosis-induced endothelial cell injury. Nevertheless, in ESRD an inverse correlation was observed between plasma ET concentrations and post-ischaemic vasodilation in an arterial territory (forearm) almost always free of atherosclerotic plaques, suggesting that the increased plasma ET in uraemic patients may be related more to generalized endothelial cell dysfunction. Increased plasma ET concentration is known to be associated with endothelial dysfunction [14,15]. Joannides et al. [55] have demonstrated that post-ischaemic dilatation of forearm arteries is mainly related to NO release by endothelial cells. They showed that post-ischaemic vasodilation of the radial artery is almost abolished in ESRD, blood flow increasing only secondary to increased flow velocity, with no increase in arterial diameter. Since direct vasodilation with nitroprusside induced a similar response in both ESRD patients and controls, authors concluded there was defective NO-mediated function in uraemic patients [55]. In ESRD patients an endogenous inhibitor of the L-arginine/nitric oxide pathway accumulates [56,57]. Inhibition of NO production could explain increased ET production, since NO inhibits ET production [58,59].

ET release is stimulated by a variety of vasoactive hormones, thrombin, inflammatory cytokines, and hypoxia [7,60–63]. While increased plasma levels of vasoactive substances and cytokines are frequently observed in haemodialysis patients and could activate the ET release, the roles of these factors were not analysed in the present study and we therefore could only speculate on their possible role.

Concluding remarks

Overall, the functional importance of circulating ET remains uncertain. The highest concentration values measured in the present study were in the range of those inducing vasoconstriction, but in the majority of patients the circulating levels of ET were below the estimated affinity (Kd) for ET receptors and were therefore unlikely to elicit physiological responses [27]. ET is predominantly an autocrine/paracrine factor and the majority of ET produced is secreted by the basolateral side of the endothelial cells, and plasma concentration may not reflect endothelial release, but rather overflow of local production [64,65]. Therefore, circulating ET could potentially be a marker of local production and it is possible that as systemic concentrations are elevated, local concentrations are even higher [66].

Finally, the inherent and necessary weakness of the present study is its cross-sectional and correlative approach which is not a proof of causal relationship. The failure to provide interventions with ET antagonists does not allow to demonstrate whether the increased plasma ET levels are the cause or the consequence of the observed cardiovascular alterations. Longitudinal clinical studies using receptors antagonists could clarify the pathogenic role of ET in cardiovascular pathology in ESRD patients.

In conclusion, the present data suggest that elevated plasma ET levels in ESRD patients are associated with chronic flow-and pulsatile pressure overload. This increase in plasma ET levels could be one mediator of mechanotransduction contributing to the cardiovascular remodelling observed in chronic uraemia.

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References


30. London GM, Pannier B, Guérin AP, Marchais SJ, Saffar ME,
Cuche JL. Cardiac hypertrophy, aortic compliance, peripheral resistance, and end-stage renal disease. Comparative effects of ACE inhibition and calcium channel blockade. Circulation 1994; 90: 2786–2796


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